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Duration of Protection From Clinical Hepatitis A Disease After Vaccination With VAQTA®

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Recent papers examining the expected persistence of anti-hepatitis A virus antibody following vaccination with inactivated hepatitis A vaccine have estimated that geometric mean antibody levels will remain above cut-off levels for 10–30 years. However, the methodology used in these papers did not take into account any estimates of variability between subjects. In this paper data from the persistence of antibody after the administration of another vaccine, VAQTA® (hepatitis A vaccine, inactivated; MSD), were used to develop further models of antibody decay. Using individual subject estimates instead of group means allowed the estimation of time to negativity for various percentiles of the population (including the median), and the construction of confidence intervals on estimates of time to negativity. Data from studies of subjects who seroreverted to negativity, and subsequently received a booster dose, were also considered to show that subjects who lose detectable antibody are likely to remain protected from hepatitis A disease by persistent immune memory and rapid anamnestic response soon after exposure to hepatitis A virus. The estimates of duration of protection suggest that VAQTA® will provide protection for many years, first through presence of antibody and further through an anamnestic response based on persistent immune memory.

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KEY WORDS: anamnestic response, antibody, hepatitis A virus, extrapolation

INTRODUCTION

Hepatitis A virus (HAV) is a leading cause of liver disease in the world. In underdeveloped countries, most children acquire lifelong immunity from primarily asymptomatic infection early in life. Persons at risk for symptomatic disease include residents of transitional developing nations, as well as travelers and military personnel visiting endemic areas, case contacts, residents of frequently affected communities, children in daycare centers and their contacts, institutionalized persons, consumers of raw shellfish and other high risk foods, and persons practicing high risk sex. While typically self-limiting, the case fatality rate is greater than 3% in patients over 49 years of age, especially if underlying disease is present [Hadler, 1991].

Prevention of hepatitis A disease can be accomplished with repeated injections of immune globulin, but each injection provides protection for only 4–6 months [Conrad and Lemon, 1987]. More recently, a single injection of VAQTA®, a highly purified vaccine made from the attenuated CR326F strain of HAV, has been shown to be efficacious in preventing clinical hepatitis A disease in a population of healthy children 2–16 years old [Werzberger et al., 1992]. A single injection induces detectable levels of antibody to HAV in most recipients within 4 weeks, while a second injection 6 months later provides high levels of serum antibody that should last for several years. Immune memory is the basis for lifelong protection from disease in people immune due to previous infection [Villarejos et al., 1982]. The presence of immune memory after a single injection of VAQTA® has been demonstrated by a sharp rise in antibody levels after a booster injection of VAQTA® [Block et al., 1993; Kuter et al., 1991; Midthun et al., 1991, 1992; Newcomer et al., 1994; Shouval et al., 1993; Nalin, 1995].

The expected duration of detectable vaccine-induced antibody to HAV has been debated. Estimated duration of antibody persistence from a different hepatitis A vaccine has been discussed in several articles [Tilzey et al., 1992; Davidson et al., 1992; Wiedermann et al., 1992; Van Damme et al., 1994]. These articles used mathemati-

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cal modeling and extrapolation procedures to find the point at which the geometric mean antibody titer (GMT) would be anticipated to cross the cutoff level (that is, the threshold level at which the assay is capable of detecting serum antibody). Based on these procedures, it was estimated that the GMT would stay above the cutoff level for 10–30 years.

The purpose of this analysis was to estimate the persistence of detectable antibody using a mathematical model based on the combined data from all available studies of persistence of anti-HAV antibody after vaccination with VAQTA®. Since the modeling/extrapolation method is subject to large variability and assumes that the same model that fits the data in the observed time range will also be applicable in the extrapolated time range, it is necessary to use the most refined techniques available in order to obtain the best estimates possible. Van Damme et al. [1994] estimated persistence of detectable antibody by assuming a particular mathematical model of the GMT over 36 months and extrapolating these data in order to estimate the time when the GMT will become negative. We believe that the method of Van Damme et al. [1994] can be improved to account for individual variation in the estimation of duration of detectable antibody so that point estimates of percentiles for the population distribution of persistence of detectable antibody may be obtained from the data, along with associated confidence intervals. In this paper a slightly more refined method that incorporates individual subject estimates of duration of antibody level was used. Using individual estimates of the length of time until each subject is predicted to become seronegative provides the ability not only to estimate the median time to loss of antibody and other percentiles, but also to allow the construction of confidence intervals on these estimates. Persistence data from the entire clinical experience of VAQTA® are used.

In addition, to explore the question of the duration of protective immune memory induced by VAQTA®, data from a study using a lower dose or a single injection in adults are presented. Since immune memory has been shown to protect from clinical disease after re-exposure to HAV of previously infected individuals, even when antibody titers have waned to undetectable levels [Villarejos et al., 1982], presence of immune memory is likely to provide an additional period of protection after antibody wanes below detectable levels. A very few vaccine recipients who had undetectable antibody levels several months after the primary vaccination were identified and offered a booster injection of VAQTA®. The immune response of these subjects after the booster injection is discussed. Studying these subjects will suggest how the immune systems of vaccinees whose antibody levels fall below the cutoff limits of the assay react upon exposure to wild virus.

EXTRAPOLATION MODEL OF ANTIBODY DECAY

Methods

Since actual persistence of antibody (time from seroconversion to seroreversion to negativity) can only be determined by the direct observation of detectable antibody over very long periods of time and no such data yet exist, persistence can only be estimated by modeling the antibody levels over the time period for which data exist and then using extrapolation. Toward this end, the VAQTA® database was searched for subjects of any age who met the following selection criteria: were seronegative before the first dose of vaccine, received any dosage, received either two or three injections with the first and last doses 6 months apart, received no booster doses after the last dose, and had serology results at 7, 12, 24 and 36 months after the first injection. This search found 118 subjects meeting these selection criteria.

All serology results used for this analysis were obtained using the HAVAB RIA assay (Abbott Laboratories, North Chicago, Illinois), modified to increase sensitivity [Miller et al., 1993]. A titer of at least 10 mIU/mL anti-HAV antibody by this assay was considered evidence of detectable antibody, and a subject with such a titer was called seropositive.

The method used in this analysis assumes the same basic exponential decline model as used by Van Damme et al. [1994] and is described by the equation:

$$Y_x = Z_n(1 - \delta)^{(x-n)/(m-n)} \quad (1)$$

where n is the number of months corresponding to the first bleed of some interval, m is the number of months corresponding to the second bleed of that interval, Z_n is the titer at month n, δ is the decrease in titer from months n to m divided by Z_n and x is the number of months until the titer reaches Y_x. This equation may be solved for x as follows:

$$x = (m - n)\left[\frac{\ln(Y_x) - \ln(Z_n)}{\ln(1 - \delta)}\right] + n \quad (2)$$

Here x is the estimated number of months until a titer of Y_x is reached. Note that Van Damme et al. [1994] used $n = 12$, $m = 24$, $\delta = 0.14$, $Y_x = 20$ and Z_n = GMT at 12 months.

The new model proposed here accounts for individual variability by calculating x on an individual basis rather than on a summary or geometric mean basis. That is, this method calculates values of δ and Z_n for each subject individually in order to estimate the length of persistence for each subject. Data from only month 24 and month 36 are used to calculate δ for each subject. The length of time to negativity is estimated for each subject using equation (2), assuming that the rate of decrease over time remains constant at the same rate of decrease observed from months 24 to 36, as illustrated in Figure 1. As a result, the distribution of the individual persistence estimates provides useful information about the variability of persistence estimates and also allows for the construction of confidence intervals for various distribution percentiles such as the median, based on order statistics [Mood et al., 1974].

To estimate the proportion of subjects maintaining antibody at future timepoints, nonparametric product-limit estimates were calculated. Instead of using the

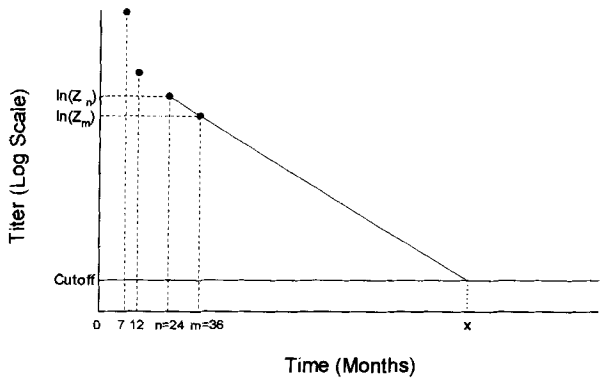

Fig. 1. Method of antibody extrapolation. Extrapolation of antibody levels, as explained in equations (1) and (2), assumes linear decay in log-transformed antibody levels starting at least as early as month 24.

TABLE I. Summary of Demographics of Study Subjects

	Dose group			
	6 U	13 U	25 U	Overall
Age class				
Pediatric (2–17 years)	5	0	22	27
Adult (at least 18 years)	29	29	33	91
Race				
Caucasian	34	28	53	115
Other	0	1	2	3
Gender				
Female	22	13	29	64
Male	12	16	26	54
Total	34	29	55	118

time when subjects actually lost antibody, however, this method used the time predicted by equation (2).

To study the effect of revaccination of subjects who lost detectable antibody, a study in which suboptimal regimens or lower doses of vaccine was used. Three hundred sixty-six adults were enrolled in a study of vaccine immunogenicity. Participants were randomized to one of five comparative groups. One group of subjects received one injection of 50 U (where 1 U ≅ 1 ng of viral protein) of VAQTA®. Three groups received 25 U vaccine per injection in two injections, with the second injection at 2, 4 or 24 weeks after the initial injection. The fifth group received 13 U vaccine, with the second dose 24 weeks after the initial dose. Immunogenicity results using various dose/schedule combinations have been reported elsewhere [Egan et al., 1993]. Twelve months after the initial dose, antibody titers were measured. Any subjects found to have antibody levels below the assay cutoff were offered a revaccination 6 months later (i.e., 18 months after the initial dose) with a single dose of 25 U vaccine. Further serology results were obtained at 1, 8 and 28 days and 6 months after the revaccination.

Results

Estimated duration of antibody. The demographic characteristics of the 118 subjects included in this analysis are shown in Table I. Subjects were divided into categories based on age in years at the time of the first injection. Those subjects 2–17 years old at the time of the first injection were called pediatric, and those subjects age 18 or older at the time of the first injection were called adult. The subjects were fairly evenly divided with regard to gender but most subjects were Caucasian adults.

Table II summarizes the information relating to percent decrease in titer for each of the three time intervals in this study: 7 to 12 months, 12 to 24 months and 24 to 36 months. (Since all 118 subjects had serology results at each timepoint, all contributed to estimates for each interval.) Medians, rather than means or geometric means, were used to summarize the data since the distributions were quite skewed. In fact, 35 of the 118 subjects actually had higher titers at month 36 than at month 24. The median antibody titer decay rate decreased over time, going from a 68% decrease in the interval between months 7 and 12 to a 19% decrease in the interval between months 24 and 36. The maximum antibody decay rate also decreased over time. The pattern held for each of the subgroups as well as the entire sample. Percent decreases in titer are also indicated in Figure 2, which shows the GMT and the median titer levels over time. The GMT and median titer had similar observed decay profiles, consistent with a two-stage model of antibody decay, with a short but steep drop coming after the peak following the last injection, followed by a slower, more gradual drop in antibody. If this pattern continues, the rate of antibody decay will slow down over time. Thus, using the rate of decay in the 24–36 month period may overestimate the decay rate, thereby underestimating the time to undetectable antibody and providing conservative estimates of antibody duration.

Figure 3 shows a scatterplot of anti-HAV antibody titers at month 24 and at month 36 for each of the 118 subjects. Isopleths show which subjects increased or decreased in titer by 25%, 50% or 75% from month 24 to month 36. No subject had more than a 75% decrease from month 24 to month 36. The rates of antibody loss were rather consistent for all antibody levels in the observed range at month 24.

Median percent decreases in titer for each time interval were relatively consistent across the various subpopulations in the table. Subjects receiving the 13 U dose had the steepest drop from months 24 to 36. However, since all of these subjects were adults, it is unclear if the cause is the dose, the age group or some other unknown factor. The subgroups were generally too small to make meaningful comparisons.

Various percentiles of the distribution of estimated length of persistence of detectable antibody are presented in Table III. These extrapolation procedures used only the observed rate of decay from month 24 to month 36, ignoring the period of more rapid decrease from month 7 to month 24. The median length of persistence of detectable antibody is estimated to be 20.9 years, with a 95% confidence interval of 14.0 to 27.0 years. Ninety percent of the population had a length of persistence of detectable antibody estimated to be at least 6.1 years, with a 95% confidence interval estimated to be from 5.4 to 7.5 years.

TABLE II. Median (Minimum, Maximum) Percent Decrease in Titer for Each Time Interval

	Sample size	Time interval		
		7–12 Months	12–24 Months	24–36 Months
6 U dose	34	64.1% (+, 88.2%)	55.1% (+, 85.4%)	23.1% (+, 71.4%)
13 U dose	29	65.7% (+, 98.8%)	58.2% (6.3%, 95.4%)	35.5% (+, 68.0%)
25 U dose	55	69.5% (+, 99.1%)	50.8% (+, 76.8%)	9.6% (+, 69.1%)
2 injections	14	78.8% (54.7%, 90.8%)	53.6% (17.6%, 72.0%)	4.8% (+, 32.4%)
3 injections	104	65.6% (+, 99.1%)	54.3% (+, 95.4%)	21.8% (+, 71.4%)
Adult	91	63.9% (+, 99.1%)	55.7% (+, 95.4%)	22.5% (+, 71.4%)
Pediatric	27	79.3% (54.7%, 92.1%)	51.5% (17.6%, 73.8%)	13.8% (+, 61.1%)
Overall	118	67.7% (+, 99.1%)	54.3% (+, 95.4%)	18.5% (+, 71.4%)

A "+" for minimum percent decrease indicates that at least one subject in the group had an increase in antibody titer level over the time range.

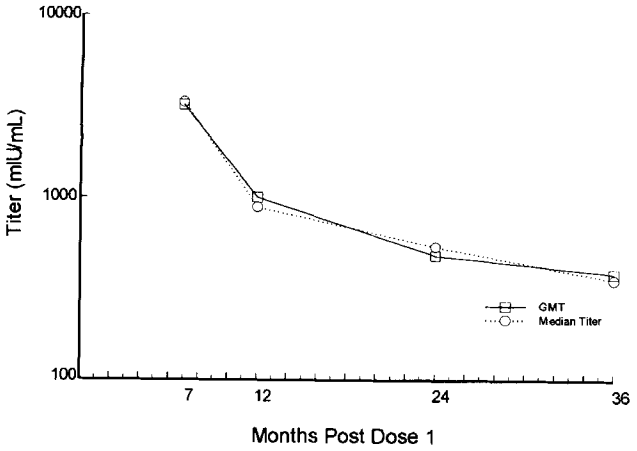

Fig. 2. Plot of geometric mean titer (GMT) and median titer over time. GMT and median titers (as measured by modified HAVAB) are very close at each timepoint. Curvature in the lines indicates that the rate of antibody loss decreases over time.

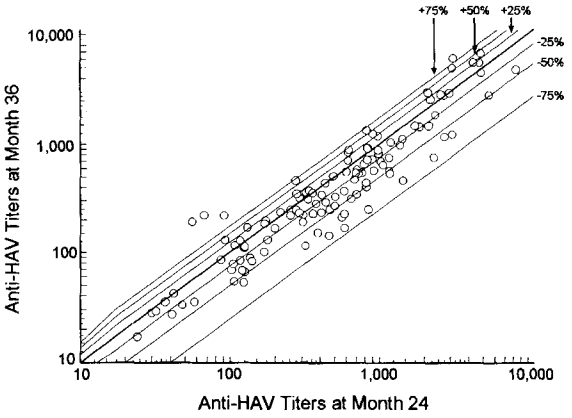

Fig. 3. Scatterplot of titer at month 24 by titer at month 36 for each subject. Isopleths show which subjects increased or decreased by 25%, 50% and 75%. No subject decreased in antibody level by more than 75% from month 24 to month 36.

Figure 4 shows the product limit estimates of the length of persistence of antibody titer, plotting the proportion estimated to be positive over time. Estimates are presented out to 30 years, at which time the model suggests that roughly 40% of the vaccine recipients will still have detectable antibody. These estimates are very sensitive to model assumptions, but still provide evidence that many vaccinees will have detectable antibody levels for many years.

Revaccination after loss of detectable antibody. In the adult revaccination study, 244 subjects were tested at month 12, with eight of those found negative. Five of the eight agreed to receive a booster injection at month 18. Table IV reports the immunogenicity results for these five subjects after the booster dose. In each case, the subject was positive within 8 days after the booster, consistent with an anamnestic response. By week 4, titers were very high, with a minimum of 623 mIU/mL, over 10 times the GMT observed 4 weeks after a single injection [Egan et al., 1993].

DISCUSSION

VAQTA® has been shown to be efficacious in preventing clinical HAV disease after a single injection. Subjects who receive VAQTA® in either two or three injections, with the last injection 6 months after the first injection, develop very high levels of anti-HAV antibody. After a peak following the last injection, these antibody levels decline rapidly for a few months, then decline more slowly over the time period of 1–3 years after the initial injection. Empirical estimates of the persistence of detectable antibody after vaccination with VAQTA® will not be available for many years. Until such data are

TABLE III. Distribution of Estimated Persistence of Detectable Antibody (in years)

Percentile	Dose group 6 U	13 U	25 U	Overall[a]
99	5.3	4.5	5.3	5.0 (4.5, 5.3)
95	5.5	5.0	7.7	5.4 (5.0, 6.0)
90	6.0	5.4	9.1	6.1 (5.4, 7.5)
75	8.2	6.3	14.9	8.6 (7.9, 11.2)
50 (median)	15.9	8.6	42.4	20.9 (14.0, 27.0)

[a]Estimated percentile and approximate 95% confidence interval.

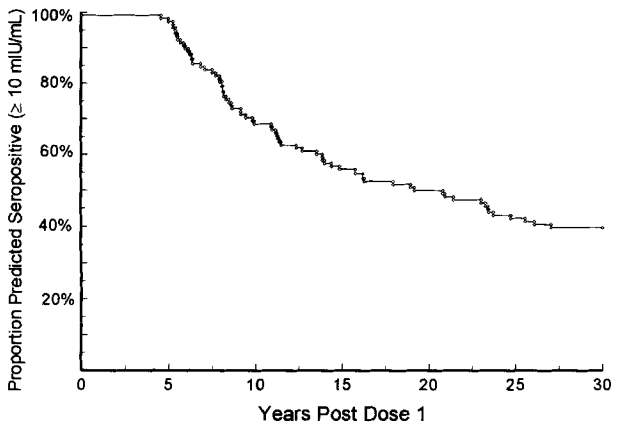

Fig. 4. Model-based estimates of proportion of vaccinees seropositive over time. Based on the model of constant antibody decay after month 24, subjects will begin to lose antibody at about 5 years post the beginning of the vaccine regimen. After 30 years, over 40% of the subjects are expected to remain antibody positive, according to the model.

available, the only mechanism to predict persistence of antibody is to use a mathematical model. By making assumptions about the rate of decline after 3 years, we have estimated that antibody levels will persist above detectable levels for many years or even decades in most subjects.

The data used for this analysis were derived from a different assay than data used by previous authors. The ELISA (enzyme-linked immunoabsorbent assay) inhibition assay (EIA) used for previous studies and the modified HAVAB assay used for this analysis are known to quantify antibody differently [Berger et al., 1993]. The cutoff for the EIA is 20 mIU/mL, so $Y_x = 20$ was used for the cutoff by Van Damme et al. [1994] in equation (2), while this analysis used $Y_x = 10$. Even though the quantification was different in the two assays, it is interesting to note that the estimated duration of detectable antibody is not markedly different between the previous results and the results in this analysis.

Models using individual values for duration, such as we have used, are much more informative than models using group means, such as those explained by Van Damme et al. [1994]. Without a measurement of the variability of a data set, assessment of the precision of any estimates is not possible. Further, estimates of percentiles are probably of more interest in the public health policy setting, since the length of time that a certain proportion of vaccine recipients are protected is more critical to policy decisions than the average duration. While the point estimates provided in previous papers after three dose regimens are not markedly different from the point estimates we have provided (and this is not surprising given that Figure 2 shows that group medians and geometric means are very close months 7 through 36), the method in this paper has the advantage of allowing for calculation of confidence limits so that an interval of likely values can be determined.

Schofield and Lei [1988] illustrated a two component bi-exponential model of the waning of antibody to hepatitis B virus surface antigen following administration of *RECOMBIVAX HB®* (hepatitis B vaccine, recombinant; MSD). After administration of the last dose of a *RECOMBIVAX HB®* regimen, antibody levels increased rapidly. After a period of rapid decrease, the decrease flattened out, so that antibody levels, while decreasing, remained relatively flat for a period of years. The methods used here to extrapolate anti-HAV antibody titers after administration of hepatitis A vaccines are consistent with such a model, but ignore the time when antibody decreases rapidly and use only data from the time when antibody decreases more slowly. While more complex models may be more useful to describe antibody response at earlier timepoints, the extrapolation of antibody from the two models will give similar results. The assumption of a two-stage model is not required for the linear extrapolation; however, only the linear pattern from month 24 to the loss of detectable antibody is required.

The decrease in antibody level may not be linear in log scale after month 36. Table II and Figure 2 both suggest that the rate of decrease may itself decrease. Figure 5 illustrates an alternative model in which the log-transformed titers decrease logarithmically, resulting in a non-linear extrapolation in log scale. (In this figure, the titers are chosen to be the group medians as shown in Figure 2, and the curve is fit to the last three points, not the last two points.) For this reason, we conclude that the methods we have used are conservative in that they may tend to underestimate the duration of detectable antibody.

Table III suggests that a higher dose of vaccine may not induce a longer duration of detectable antibody. Confounding between the dose and the ages of the recipients (pediatric or adult), the number of injections received (two or three) or the study site does not seem to explain this pattern. Further, higher doses were not always associated with higher antibody responses (GMTs) to the original vaccination series in studies reported by Newcomer et al. [1994] and Block et al. [1993]. While this observation may be the result of over-analyzing a limited amount of data, the relationship between the dose of vaccine received and the duration of detectable antibody warrants further study.

The incubation period of HAV is thought to be 14 to 50 days, being longer in children than adults [Ward et al., 1958]. An anamnestic response that begins within a

TABLE IV. Response to a Booster Dose in Adults Who Were Seronegative at 12 Months After the First Injection

Case number	Dose and schedule	Antibody titer after revaccination			
		Day 1	Day 8	Week 4	Month 6
234	50 U in one injection	<10	28	623	ND
257	50 U in one injection	<10	99	1927	1272
319	50 U in one injection	<10	348	1031	218
326	25 U at 0 and 2 weeks	<10	80	873	ND
327	25 U at 0 and 4 weeks	ND	161	2218	1078

ND: No data available for this subject at this timepoint.

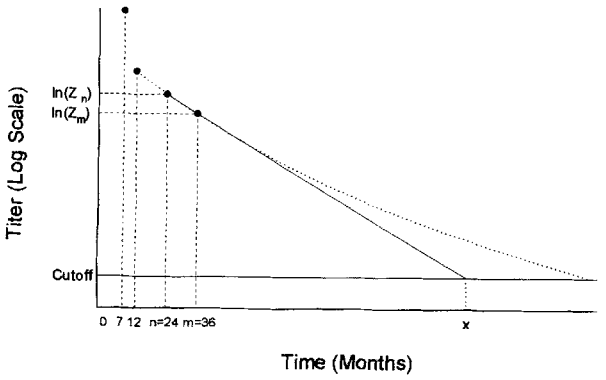

Fig. 5. Alternative methods for extrapolating antibody levels. If the rate of antibody decay slows over time (in log scale), the estimated duration of detectable antibody will change. This figure shows projected antibody levels assuming a log-log decrease (dotted line) and assuming a linear decrease (solid line).

week of exposure to the virus and peaks within a month (as shown in the adult revaccination study) should provide some protection against hepatitis A disease in adults long after vaccination, even in the absence of detectable circulating antibody. Nalin [1995] published results of a revaccination study in children and adolescents who had lost detectable antibody 6, 12 or 18 months after a single 25 U injection of VAQTA®. Among those children who received a booster 18 months after the first injection, all responded anamnestically to a 25 U revaccination with at least 2,005 mIU/mL by modified HAVAB assay. Overall, the child with the lowest antibody response, a child who received a booster 6 months after the first dose, responded with a titer of 1,686 mIU/mL, about 40 times the GMT after the first dose in that study [Werzberger et al., 1992]. It seems logical to conclude, then, that even when antibody wanes below detectable levels, immune memory is still intact providing even longer protection from wild virus. Thus, a two dose vaccination series of VAQTA® (with the second dose given 6 to 18 months after the first) should provide protection against clinical hepatitis A disease for many years after the series is complete, and even after loss of detectable antibody. (The duration of antibody persistence and immune memory after a single dose of VAQTA® awaits future study.)

A key issue yet to be resolved involves recommendations regarding the timing of extra booster doses of vaccine in subjects likely to have lost detectable levels of antibody. Villarejos et al. [1982] showed that disease-induced protection is due to immune memory. If vaccine-induced protection is also due to immune memory, and if that immune memory remains intact long term in vaccine recipients, then, based on the data in Table IV and the data presented by Nalin [1995], exposure to wild virus should trigger an anamnestic response early enough after exposure to prevent clinical disease through the relatively long incubation period. In that case, booster doses may not be necessary. However, if vaccine-induced protection is based on circulating antibody, then booster doses should be recommended. Although the data presented here suggest that such a booster dose may not be needed for at least 5 years in most subjects, further study is required before a recommendation on the timing of such booster doses is made.

Any conclusion made from data and models described in this paper must not be considered definitive. As more data accumulate over the next few years, further analysis of the kinetics of antibody decay will undoubtedly take place. Only when the time comes that vaccine recipients start to lose detectable antibody will precise estimates of duration of antibody be made without the necessity of unverifiable assumptions. As this time is approaching for the hepatitis B vaccines that have been available for over a decade, and as the model in this paper predicts a long duration of detectable antibody in recipients of VAQTA®, observation of actual measured loss of antibody in VAQTA® recipients will require continual study, as will the comparisons of lifelong protection based on immune memory alone in vaccinees versus individuals infected early in life.

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